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Research Article

Acute and sub-acute toxicity study of *Trema orientalis* (L.) Bl. methanol extract in rats

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ABSTRACT

The medicinal plants find wide applications in pharmaceutical, cosmetic, agricultural and food industry. *Trema orientalis* has emerged as a good source of phytomedicine. *T. orientalis* has beneficial therapeutic properties, and indicates that it has potential as an effective herbal remedy for several diseases. The present study was undertaken to evaluate the acute and sub-acute toxicity of methanol powder extract of aerial part of *Trema orientalis*. There were no major changes in the body weight of animal treated with plant extract. The biochemical parameters like ALP, SGPT, SGOT, total protein, globulin, albumin and bilirubin were within the normal limit in the plant extract treated groups of animals. The present study shows that the methanolic extract of *Trema orientalis* is safe in lower dose for pharmaceutical analysis.

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INTRODUCTION

Pharmacological evaluation of medicinal plants has recently witnessed a growing interest amongst researchers worldwide. Research on the therapeutic potential of plants has surged over the years, with volumes of scientifically documented information showing considerable potential for medicinal plants to be used in the treatment of several diseases¹. However, while voluminous pharmacological studies have been conducted to ascertain the subjective traditional uses of various medicinal plants, very few plants have been thoroughly evaluated for their detrimental effect. Reports of efficacy are, by far, more numerous than those on toxicity^{2,3}. There is, therefore, a need to further the investigation the toxicological effects of medicinal plant extract intended to be used in animals or humans is a crucial part of its assessment for potential toxic effects.

The Ulmaceae family consists of 15 genera and 200 species are distributed over tropical and temperate regions of the northern hemisphere^{4,5}. Most of the species in this family are of evergreen or deciduous trees and shrubs. Few plants of this family are medicinally evaluated for Pharmacognostic and Phytochemical analysis. Still, many potent plants of this family were not revealed by the scientific world so far.

Perusal of the previous literature revealed that medicinal plant *Trema orientalis* (L.) Bl. is unexplored for its detailed systematic acute and sub-acute toxicity studies. The present study was undertaken to evaluate the *Trema orientalis*.

Traditional uses of *Trema orientalis*

Food: The leaves and fruit are reported to be eaten in the Democratic Republic of Congo.

Fodder: The leaves, pods and seeds are used for fodder. Silage made from the foliage has a crude protein content 18.9 g/100 g dry matter, and in the Philippines is fed to cattle, buffaloes and goats. The high fibre content and toxins usually limit the use of leaf meal in feeds.

Medicine: The leaves and the bark are used to treat cough, sore throats, asthma, bronchitis, gonorrheas, yellow fever, toothache, and as an antidote to general poisoning⁶. Some pharmacological research done on the plant has focused on, hypoglycaemic activity, analgesic, anti-inflammatory activities, anti-plasmodial activity, diuretic activity, laxativity effect, anti-convulsant activity, anti-helmintic activity, anti-sickling effect, anti-oxidant, and anti-bacterial activity⁷. The leaves are reported to be a general antidote to

poisons and a bark infusion is drunk to control dysentery. A leaf decoction is used to deworm dogs ⁸.

Methanol extract of *T. orientalis* possesses strong antioxidant activity ⁹. This activity may be due to the presence of phenolic compounds (tannins and flavonoids) present in the extract. The presence of tannins, saponins, flavonoids and triterpenoid are reported in methanolic extract of *Trema orientalis* ¹⁰.

MATERIALS AND METHODS

Preparation of the extract

Trema orientalis aerial part were collected, shade dried and crushed to coarse powder. The air dried powdered plant material was taken in a Soxhlet apparatus and extracted with methyl alcohol till the extracts became colourless. 250 g of coarse powder was mixed with 50 ml of methyl alcohol and refluxed for 3 hr. Methyl alcohol extract was concentrated.

Experimental animals

Wister albino rats (20 - 40 g) used in the present study were housed in polypropylene cages (38 x 23 x 10 cm) with not more than six animals per cage under standard environmental conditions of temperature ($23 \pm 1^\circ\text{C}$), relative humidity ($55 \pm 1\%$), 12 h/12 h light/dark cycle and fed with standard pellet diet (Pranav Agro Industries Ltd., Sangli, India) and water *ad libitum*. The animals were acclimatized to the environment for two weeks prior to experimental use. Animals were fasted over night before the experimental schedule, but had free access for water *ad libitum*.

Experimental design

Acute oral toxicity studies were performed according to OECD (Organization for Economic Co-operation and Development) guidelines ¹¹. Wister albino male rats (n = 6/each dose) selected by random sampling technique were employed in this study. The animals were fasted for 12 h with free access to water only. The sample extract of the selected plant species was administered orally at a dose of 5 mg/kg initially to rats and mortality was observed for 7 days. If mortality was observed in 4/6 - 6/6 animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one rat out of six animals then the administration of the dose was repeated with higher doses such as 1000, 2000, 3000 and 4000 mg/kg. The mortality and clinical signs, which includes changes in skin, fur, eyes and mucous membranes, were observed for the first 4 h subsequently for 72 h and thereafter for 7 days of test drug administration. The gross behaviours like body positions, locomotion, rearing, tremors and gait was observed. The effect of plant extracts on grip strength, pain

response and righting reflex were observed. The daily food and water intake, and body weight changes were also monitored.

Sub Acute toxicity

The study was conducted in compliance with OECD guidelines. The experimental animals were divided into four groups of 6 rats each. The groups were treated daily with three doses of ASME (250, 500 and 1000 mg/kg b.w.) for 28 days. All treatments were administered via oral gavage.

Observation

Clinical signs were observed at least twice a day during the 28-day treatment period. Body weights were measured once a week. On the 29th day, the animals were fasted overnight and blood samples collected via cardiac puncture. Hematological analysis of the blood samples was performed using an automatic hematology analyzer. The parameters which were evaluated included: red blood cells (RBC) count, hemoglobin (Hb), leukocytes (WBC) count and eosinophils counts. For biochemical analysis purposes, the blood samples were centrifuged at 3000 rpm for 15 min. Diagnostic kits were used to evaluate these parameters, which included the serum levels of Total Proteins (TP), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), glucose, bilirubin, Alkaline Phosphatase (ALP), serum creatinine, uric acid, urea and the rats' lipid profiles, i.e., the levels of Total Cholesterol (TC) and Triglycerides (TG). Histopathological examination was also conducted on the liver, lungs, brain, kidney and heart of the treated control groups ^{12, 13}. The values obtained for the control group were considered as the reference values and statistical analysis was conducted against the control group.

Statistical analysis

All the data were expressed as mean \pm SEM (n=6). The mean of the different groups was compared using one-way analysis of variance.

RESULTS AND DISCUSSION

Acute oral toxicity effects of selected plants

There were no animal deaths in the first set of three male rats receiving 2000 mg/kg of *T. orientalis* methanolic extracts. No sign of toxicity was observed in the wellness parameters during the 14-day observation period. A similar observation was made in the second set of male rats treated with 2000 mg/kg of the extract. Therefore, the approximate acute lethal dose (LD₅₀) of *T. orientalis* methanolic extract in male rats was estimated to be higher than 2000 mg/kg (Table: 1).

Table 1: Effect of treatment with methanolic extract of *T. orientalis* on the acute toxicity

S. No	LD ₅₀ or MTD (mg/kg)	ED ₅₀ or 1/5 th of LD ₅₀ (mg/kg)	ED ₅₀ or 1/10 th of LD ₅₀ (mg/kg)
1	2000	400	200

MTD : Maximum tolerated dose

LD₅₀ : Lethal dose producing lethal effect in 50% population

ED₅₀ : Effective dose producing pharmacological effect in 50% population.

Cage side observations did not record any behavioural changes such as tremor, convulsion, salivation, diarrhea, lethargy or sleep during the first four hours of chosen extracts (400 mg/kg body weight) administration. Acute toxicity study recorded zero mortality at the end of 24 h

period, following plant methanolic extract administration. No behavioural alterations are recorded during the first four hours after administration of plant extracts. Hence, the LD₅₀ of methanolic extract of *T. orientalis* is thought to be greater

than 2000 mg and therefore plant extract can be considered as non-toxic upto the said dose.

Sub-acute oral toxicity effects of selected plant

Effect of Oral Administration of *T. orientalis* extracts on Body Weights

The gain in the body weight of the *T. orientalis* extracts are presented in the Table 2. The sub-acute toxicity study, which

involved rats given *T. orientalis* orally at doses of 400 mg/kg/b.w. demonstrated significant changes in animal behaviour, as well as significant reductions. There is a gradual increase in the body weight values from the day 1 to 28th day in all samples (Table 2). 4.8 g increase in weight is observed in the control sample but in *T. orientalis* increase in 6.2 g.

Table 2: Body weights (g) of the rats in the sub-acute toxicity study of the methanolic extract of *T. orientalis* (Results were expressed as the mean of 6 rats)

Group No.	Drug treatment	Dose (mg/kg) p.o.	Body weight (g)				
			1 st day	7 th day	14 th day	21 st day	28 th day
1	Control (normal saline)	10 ml/kg p.o.	121.3	122.5	123.4	124.4	126.1
2	<i>T. orientalis</i>	400	125.3	126.3	127.6	128.1	131.5

Effect of oral administration of *T. orientalis* extracts on serum biochemical parameters

The effects of administration of methanolic extract of *T. orientalis* to rats on the alkaline phosphatase (ALP), serum glutamate oxaloacetate transaminase (SGOT), Serum

glutamate pyruvate transaminase (SGPT) and bilirubin content is shown in Table 3. Increased ALP, total protein and SGPT levels were observed in both the selected species in the groups treated with 400 mg/kg compared with the control group ($p < 0.05$). In *T. orientalis* extract the level of bilirubin and SGOT are mildly reduced, when compare with control.

Table 3: Biochemical parameters in the serum of rats orally treated with the methanolic extract of the *T. orientalis* (Results were expressed as the mean of 6 rats)

Group No.	Drug Treatment	Dose (mg/kg p.o.)	Albumin (g/dL)	Globulin (g/dL)	Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Indirect Bilirubin (mg/dL)	Total Protein (g/dL)	SGPT (Units/L)	SGOT (Units/L)	ALP (Units/L)
1	Control (normal saline)	10 ml/kg p.o.	4.21	4.51	0.27	0.11	0.12	7.56	41.32	88.91	137.8
2	<i>T. orientalis</i>	400	4.97	4.91	0.26	0.10	0.11	8.21	47.50	88.21	140.2

Albumin was significantly increased in *T. orientalis* extract treated groups ($p < 0.01$) (Table 3). Moreover, a mild increase in serum globulin levels was observed in the rats treated with 400 mg/kg of *T. orientalis* extract. In the male treated rats, total bilirubin levels were slightly decreased in *T. orientalis* extract treated groups. The total protein (g/dl) content and glucose (mg/dl) content also increased in selected extract as compare with the control.

By the transfer of an amino group, Aminotransferase SGPT and SGOT catalyze the interconversion of amino acids and α -keto acids. These enzymes are very sensitive and are reliable source for hepatoprotective and curative effects of various compounds¹⁴. Bone, liver, intestine, placenta produce Alkaline phosphatase (ALP) and is also excreted in the bile. There is an elevated serum ALP levels due to increased production of ALP by hepatic parenchymal or duct cells in the absence of bone disease and pregnancy¹⁵. Bilirubin (a metabolic product of the breakdown of heme level) increases in diseases of hepatocytes, obstruction to biliary excretion into duodenum or in hemolysis¹⁶. Serum enzyme levels are not a direct measure of hepatic injury. But elevated levels are

indicative of cellular leakage and loss of integrity of cell membrane. When the enzyme content in serum is lowered, it indicates the hepatoprotection of the drug. The results are further supported by histopathological studies that the *T. orientalis* extracts can be used as a potential hepatoprotective drug and did not exhibit any toxicity.

Effect of oral administration of *T. orientalis* extracts on serum electrolytes

Estimation of serum electrolytes urea and creatinine show significant variation between control and methanolic extract treated groups of *T. orientalis* (Table: 4). Abnormally high levels of serum creatinine, uric acid and urea are biomarkers of possible malfunction of the kidneys¹⁷. In this study, both urea and creatinine levels are marginally altered in treated rats compared with their respective controls. The changes in the blood urea level parallel with changes in the blood creatinine level. Nevertheless, the values are within the normal ranges of these parameters, which ruled out the possibility of precipitated abnormalities. Thus, these findings suggest that *T. orientalis* powder methanolic extract does not affect the normal kidney function.

Table 4: Serum electrolytes values of the rats in the sub-acute toxicity study of the methanolic extract of the *T. orientalis* (Results were expressed as the mean of 6 rats)

Group No.	Drug Treatment	Dose (mg/kg p.o.)	Urea (mg/dL)	Creatinine (mg/dL)
1	Control (normal saline)	10 ml/kg p.o.	40.21	0.78
2	<i>T. orientalis</i>	400	46.21	0.88

Effect of oral administration of *T. orientalis* extracts on plasma hematological parameters

Analysis of blood parameters in animal toxicity studies is important to report alterations in those parameters and evaluate the relative risk to the hematopoietic system when extrapolating those findings to humans^{18, 19}. Determining certain blood biochemical parameters and investigating major toxic effects on specific tissues, specifically the kidneys and the liver, may provide useful information regarding the mechanisms of toxicity of an otherwise safe and therapeutic agent²⁰.

Haematological parameters of *T. orientalis* dried powder extracts are analysed and reported in Table: 5. The level of plasma haemoglobin and esonophil (ESR) slightly increase in *T. orientalis* extracts compare with the control. Significant decrease in the level of white blood cells (WBC) and red blood cells (RBC) count are also observed in *T. orientalis* extracts. The decline in the count of WBC may suggest that reduction, immune system, rendering animals more susceptible to disease. The increase in haemoglobin suggested that *T. orientalis* extracts may reduce anemia.

Table 5: Serum haematological parameters of rats orally treated with the methanolic extract of the *T. orientalis* (Results were expressed as the mean of 6 rats)

Group No.	Drug Treatment	Dose (mg/kg p.o.)	Haemoglobin gm %	WBC 10 ³ /cu.mm	RBC 10 ⁶ /cu.mm	ESR mm/1 st hr
1	Control (normal saline)	10 ml/kg	14.21	8.11	6.21	3.12
2	<i>T. orientalis</i>	400	14.88	7.91	6.01	3.41

Effect of oral administration of *T. orientalis* extracts on plasma glucose level and lipid profile

There is increase in levels of glucose, cholesterol and triglycerides in *T. orientalis* extracts compared with the

control groups (Table 6). The lipid profiles of the treated rats demonstrated significantly increased HDL levels. HDL is known to be the good cholesterol in the body as it facilitates the prevention of cardiovascular risk factors. The triglycerides and glucose level also marginally increased.

Table 6: Effect of oral administration of the methanolic extract of the *T. orientalis* extract on plasma glucose levels and lipid profiles in rats (Results were expressed as the mean of 6 rats)

Group No.	Drug Treatment	Dose (mg/kg)	Cholesterol (HDL) (mg/dL)	Triglycerides (mg/dL)	Glucose (mg/dL)
1	Control (saline)	10 ml/kg p.o.	80.3	20.23	80.6
2	<i>T. orientalis</i>	400	84.53	21.24	86.3

CONCLUSION

The results have shown the methanolic extract of *T. orientalis* in lower doses no possible damage in the vital organs.

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